THE STRUCTURE AND BEHAVIOR OF *ACTINOBOLUS VORAX* N. SP. (PROTOZOA, CILIATA).

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Actinobolus radians was first described briefly by Stein in 1867 and since then a number of observers have given descriptions of what they considered to be the same species. Thus, Entz ('82), Erlanger ('90), Calkins ('01), Moody ('12), Penard ('22) and Fauré-Fremiet ('24) have all described what they have called *Actinobolus radians*. But a review of their descriptions and illustrations makes it apparent that they were not all dealing with the same species. However the matter of the identity of the several species represented in the literature will not be dealt with here. It is sufficient at this time to point out that the species now to be described is different from any of those indicated in the available literature, and is therefore given a new name.

It is the purpose of the present paper to record some observations on the structure and behavior of this new species of *Actinobolus* which was found in the pond of the Botanical Gardens of the University of Pennsylvania. This ciliate appeared in considerable numbers in this pond during May, 1926. A number were fixed in various fixatives, chiefly Schaudinn's sublimate-alcohol-acetic and Bouin's picro-formol-acetic. Some were stained in toto with hæmalum and other stains, while others were sectioned and stained in a variety of ways but chiefly with Heidenhain's iron-alum hæmatoxylin.

Drawings represented by figures 1, 3 and 4, were made by Mr. R. M. Stabler to whom great credit is due for his care in executing them. I am also indebted to the Pennsylvania chapter of the Society of Sigma Xi for a grant of funds which made it possible to employ Mr. Stabler to make the drawings.

Actinobolus vorax is rather large, most specimens measuring between 100 μ and 200 μ in length with a width from ½ to ¾ as great. The form varies from an elongate oval to spheroid. The more elongate individuals are narrower and more tapering

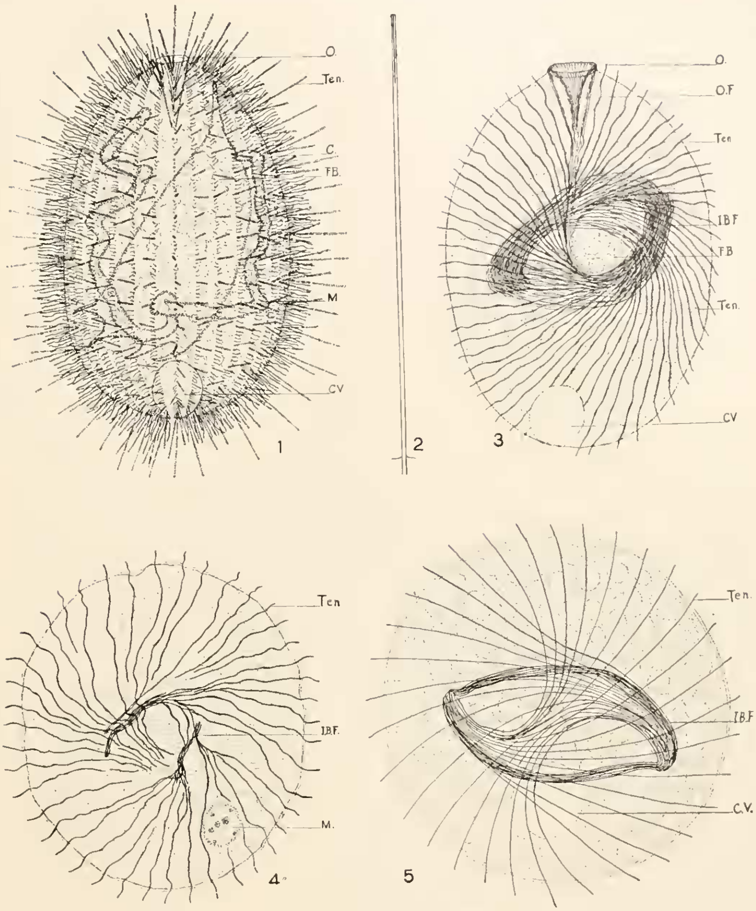

FIG. 1. Diagrammatic figure of *Actinobolus vorax* showing details of organization and the inclusion of an ingested *Anurea.* Tentacles partly extended. FIG. 2. Single tentacle, fully extended; toxicyst at distal end. FIG. 3. Side view showing inner extensions of tentacles and their association with the skein of internal fibrils. FIG. 4. Cross section of *A. vorax* showing tentacles and inner bundles of fibrils as seen in a thin section. FIG. 5. Whole animal viewed from posterior end; partly diagrammatic, showing bilateral arrangement of tentacles and their inner extensions connected with the skein.

EXPLANATION OF FIGURES.

All figures have been drawn at a magnification of 1000 and have been reduced about 3/5 in printing.

ABBREVIATIONS.

C.—cilia.	M.—macronucleus.
C. V.—contractile vacuole.	O.—oral opening.
F. B.—food body.	O. F.—oral fibrils.
I. B. F.—inner bundle of fibrils.	Ten.—tentacles.

anteriorly and more rounded posteriorly (Fig. 1). They are commonly a light yellowish brown in color. As in most other members of the family Enchelinidæ, the body is approximately radially symmetrical about its long axis with a mouth at the anterior pole and a cytopyge at the posterior pole.

Figure 1 indicates the general morphology of *Actinobolus vorax* with the tentacles only partially extended. This specimen is 110 microns long by 70 microns wide. The mouth at the more tapering anterior end does not usually protrude much beyond the general contour in the normal living animal but often protrudes slightly in fixed and stained animals, as indicated in the figure. Here it is about ten microns wide but the width varies in different specimens. The mouth is followed by a pharyngeal apparatus which is conical in shape, with the narrower end of the cone reaching into the body a distance of 20 to 25 microns. There are two sets of pharyngeal fibrils, an outer and an inner set. These fibrils do not seem to be of the nature of trichites. The inner group appears to be a thicker one, containing more fibrils and the outer set may include tentacles in its make-up as seen in some of the sections. The two sets of fibrils appear to converge at their outer extremities in what may be called the lip of the cytostome. In this lip there is a circular strand of more deeply staining material which may possibly be a sphincter for closing the mouth or may possibly be some part of a neuromotor system. In some stained specimens a second circular strand is seen a short distance outside the one just mentioned. Just how the two sets of longitudinal fibrils function is not known, but if they are contractile, the outer set might serve to open the mouth and the inner set serve to help close it or perform peristaltic movements which would aid in swallowing. While these animals were never seen to ingest anything except the rotifer, *Anurea*, stained specimens have, at times, revealed within them euglenoids, both encysted and not encysted, and several kinds of diatoms. In figure 1 an ingested *Anurea* is indicated.

At the posterior end one finds the large contractile vacuole (c.v.) which is usually subterminal (Fig. 3), and near it the terminal cytopyge which is not visible except during defecation and is not illustrated in the drawings. In many specimens there is a smaller contractile vacuole on one side a little anterior to the middle of the body. It is not certain whether it is always present or may merely anticipate the next division. It has been noted more frequently in the more elongated specimens, but in stained animals it is seen in those in which there was no evident preparation for division.

The protoplasm is rather uniformly vacuolated or alveolar and contains the long, rope-like macronucleus which may be irregularly bent, is sometimes branched, and commonly makes a complete loop within the body, with the free ends at the anterior part of the animal (Fig. 1). Micronuclei were not definitely made out, although some small bodies were found which were tentatively identified as micronuclei.

The long cilia are arranged in from 30 to 60 longitudinal or slightly spiral rows. The tentacles are in the rows with the cilia, there being about thirty in each row. Thus the cilia are not arranged in groups about the bases of the tentacles, as described by Erlanger ('90), Calkins ('01), Moody ('12) and Penard ('22). The tentacles are fairly uniformly spaced through the mid-body region but less so toward the ends. There are usually from four to eight cilia between two adjacent tentacles in a row (Fig. 1).

The most interesting morphological character of *Actinobolus* consists in the series of tentacles which appear to be so unusual among the Ciliata although true tentacles are characteristic of the Suctoria. These tentacles are capable of being extended out in all directions to a distance as great as twice the diameter of the animal, yet may be withdrawn till they no longer protrude above the body surface. They are thus customarily withdrawn in actively swimming specimens but if a moving *Actinobolus* be followed for a time it will usually not be long till the tentacles gradually emerge from the surface and as they become longer and longer the swimming activities of the cilia gradually diminish till the animal comes to rest with the tentacles fully extended. After a few seconds or minutes the cilia become more active while the tentacles begin to shorten and soon *Actinobolus* begins swimming forward while the tentacles complete the process of re-entering the body.

The relatively long tentacles of this *Actinobolus* are uniform in diameter and at first appeared to be homogeneous throughout.

However, more careful study has revealed a highly refractive region within the outer portion for a distance of about ten to twelve microns from the tip (Fig. 2). This highly refractive rodlet is believed to be a trichocyst of the chemical type and therefor may be referred to by Visscher's ('23) term, toxicyst. The species of *Actinobolus* under consideration fed primarily upon the rotifer, *Anurea cochlearis* and the paralyzing effect of these toxicysts on *Anurea* is very evident whenever one of them comes into contact with the "forest of tentacles" presented by *Actinobolus*. One may therefore consider the tentacles as devices for extending the toxicysts out from the body, increasing thereby the area of possible contact between the ciliate and its prospective prey.

Each toxicyst is thus placed at the outer end of a relatively long and retractile stalk. But what becomes of the stalks when they are retracted into the body? What is the nature of these stalks? Are they composed of material that dissolves or changes to the sol state as they retreat inwardly, to be reformed when they emerge again, or are they permanent structures which must be accommodated within the animal's body? If the latter, how are they managed; what causes them to be withdrawn; what causes them to be extended? Have they extensile and contractile properties within themselves or do they wind up in some way as on a spindle or windlass when retracted and unwind when they are extended?

The inner structure of *Actinobolus vorax* as revealed by staining both entire animals and sections indicates that the stalks of the toxicysts are permanent structures that are accommodated within the animal when they are withdrawn.

In a specimen stained entire with hæmalum a group of fibrils was noticed in the interior of the animal arranged as shown in figure 3. Careful examination revealed that the tentacles could be traced inward from the surface of the body to join with an inner skein-like arrangement of fibrils. These findings were confirmed by the study of many other specimens both mounted entire and sectioned.

For example, Fig. 5 illustrates another individual as seen from the posterior end. This drawing is somewhat diagrammatic in that only enough of the tentacles and inner fibrils are shown to illustrate the general features of the arrangement. Fig. 4 represents a cross section outlined under the camera lucida. Apparently this section is viewed from the anterior end. Here, as in the two other drawings the inner extensions of the tentacles are seen to converge into two fibrillar bundles. The following statements will refer primarily to figure 3 since the entire system is more completely illustrated by this drawing. Here it will be seen that the inner extensions of the tentacles of the left side of the animal merge into the more posterior portion of the skein and converge at the right hand angle of the skein. From this angle a series of inner fibrils extend around and across the body by a somewhat anterior route to the left hand angle of the skein, being joined by the inner extensions of the tentacles from the right side of the body. From this left hand angle a bundle of fibrils extends around and joins to the right hand angle of the skein, thus completing the system.

There can be recognized four parts to this integrated system of fibrils: (*a*) a peripheral portion consisting of (1) the tentacles with their inner extensions from left side of the animal which converge to a point on the opposite side, and (2) the corresponding set of tentacles with their inner extensions from the right side of the animal; and (*b*) the anterior and the posterior groups of connecting fibrils which complete the skein. With such an arrangement it is difficult to avoid the impression that withdrawal of the tentacles would be accompanied by a winding up of the skein and extension of the tentacles would be accompanied by an unwinding movement. However, it is entirely possible that the extension and withdrawal of the tentacles may be due to extensile and contractile properties within the tentacles themselves.

A system of fibrils connecting with the cilia has not been made out but the inner ends of the pharyngeal sets of fibrils do seem to be connected with this system (Fig. 3).

The system of fibrils just described cannot be thought of as rigid in structure nor constant in position, although the general relationships are doubtless persistent. When one realizes that *Actinobolus vorax* will swallow such a relatively large object as a rotifer (*Anurea,* as in Fig. 1) and that room is sometimes made for two or three of these food bodies, it will be realized that this system of fibrils must accommodate itself to these relatively large masses of food. In whole mounts of some specimens, the skein can be seen to be wrapped tightly around ingested Anureas.

In connection with the arrangement of the inner connecting fibrils and their accommodation to varying amounts of food, it may be noted that, as shown both in the sideview and polar views, the tentacles do not usually extend out in a strictly radial direction. When a living specimen is viewed from either pole, it can be seen that the tentacles extend out at such an angle as to indicate that their inner extensions pass somewhere between the center and the periphery of the body. In the living animals it is also noticeable that not all the tentacles extend out to the same distance from the body, some appearing to be shorter than others. It may be supposed that food bodies within the animals would interfere with the full extension of some of the tentacles.

Altogether, the evidence indicates that the tentacles are permanent structures, each bearing a toxicyst at its outer end, and also so connected and arranged within the animal to make an integrated system of fibrils, which presumably function in such a manner as to bring about the extension of the tentacles and their complete withdrawal.

Behavior.

The behavior of *Actinobolus vorax* is highly interesting as is that of all the species of *Actinobolus* so far described. If one chances upon one of these ciliates with its tentacles fully extended and then watches patiently it will usually not be long before the cilia become more active and the tentacles begin their retreat into the body. This withdrawal of the tentacles is gradual but takes only a few seconds. By the time the tentacles have been withdrawn about two thirds of their length, the cilia may be active enough to start the animal slowly on its way. Also one may see that the tentacles can be caused to wave about somewhat by the beating of the cilia, but they do not become bent in the process. As the ciliary activity increases, the animal gathers momentum and the tentacles complete their retraction so that they may be completely out of sight by the time the individual is going at full speed. In specimens that do not appear to be normal, the retraction of the tentacles may not be entirely completed and such individuals may swim about with the tentacles partly protruding from the surface. As *A. vorax* swims forward it describes a spiral path and rotates on its long axis.

After a specimen has wandered about for a time, the tentacles begin to emerge again while the speed of progression gradually diminishes; and the longer the tentacles become, the slower is the swimming movement till the tentacles are fully extended and the individual is at rest, with its long axis horizontal. During this period of relative quiescence the cilia continue to move fitfully and sluggishly, but usually with the effective stroke toward the anterior end. After another period of quiescence, which may last for a matter of seconds or many minutes, the tentacles are again withdrawn and the animal swims away to come to rest in some other place. These alternate periods of swimming and quiescence are continued indefinitely and constitute the normal round of activity of this species.

If, now, while *A. vorax* is in its quiescent state with its tentacles extended out in all directions, a rotifer of the genus *Anurea* should swim against the tentacles, one would observe that the rotifer stops all movements as if completely paralyzed. The *Anurea* is then gradually drawn to the surface of the ciliate and is passed to the anterior end where it is brought in front of the mouth which expands sufficiently to engulf this relatively large food mass. All this indicates that the tips of the tentacles contain a paralyzing substance (in the toxicysts), that retraction of the tentacles draws the prey toward its captor and that either by the activity of the tentacles or the cilia, or possibly by both combined, the prey is carried forward to the mouth which opens and swallows the prey.

Watching the activities of *Actinobolus vorax* one is reminded of a fisherman who goes fishing with a copious supply of tackle. This fisherman wanders about till he sees a likely-looking place, then sets out all his lines and waits for the fish to "bite." If, after waiting till his patience is exhausted, he gets no "bites," he takes in all his tackle, goes in search of another promising spot and again puts out all his lines, repeating the process over and over again.

Actually, of course, *Actinobolus* does not *see* anything and makes no choice of a "fishing location." Under normal conditions of the environment, the observed behavior constitutes its normal method of obtaining food; and since this behavior contributes to the welfare of the animal it may have the appearance of being purposeful. However, this behavior must be considered to be as automatic as any reflex action of a higher animal. If the environment becomes unfavorable, the behavior may be changed and the animal may swim indefinitely without coming to rest to "go fishing." Here again, its behavior contributes to the welfare of the animal, since continued swimming is more likely to bring it into a more favorable environment than would quiescence.

Discussion.

It is a little surprising that the internal structure of *Actinobolus* has not heretofore been described, but all the authors mentioned in the introduction, except Moody ('12) limited themselves to the study of animals in the living condition or else in temporary mounts after treatment with various agents.

The only internal structure mentioned by Stein ('67) was the nucleus. Entz ('82) stated that the tentacles could not be followed into the body but that when they are withdrawn they appear to vanish completely; and he found no trace of them after the use of reagents. Erlanger ('90) could make out the refractive trichocysts in the body after the tentacles were withdrawn, and could follow the inner part for some distance when the tentacles were partially extruded. Calkins ('01) does not mention the inner part of the tentacles but Moody ('12) traced them into the cortical region but not into the "endoplasm." Penard ('22) thought he could see the trichocysts imbedded in the cytoplasm when the tentacles were withdrawn, and Fauré-Fremiet ('24) reported and figured the tentacles as being visible for some distance into the body. However, none of these authors followed the tentacles inward to their connection with an internal system of fibrils, such as the present study has revealed.

Entz and Fauré-Fremiet described the cilia as arranged in rows as I have found them in *A. vorax,* while Erlanger, Calkins, Moody and Penard describe them as arranged in rows of clumps, each clump consisting of several cilia surrounding a tentacle. The animals with the cilia in clumps can scarcely be thought of as belonging to the same species as those with the cilia singly in rows. However, the animals described by Entz and by Fauré-Fremiet are so different in other respects from *A. vorax* that they can not be considered to be the same species.

It may also be noted that the feeding habits of the kinds of *Actinobolus* heretofore described are different in detail from those of *A. vorax*. Thus, Entz thought the kind described by him was capable of dissolving the cellulose walls of filamentous algæ and then devouring the cell contents. Calkins and Moody report that the *Actinobolus* studied by them "fished" always with its mouth downward and fed only on Halteria. *Actinobolus vorax* rests with the long axis horizontal and feeds primarily on *Anurea cochlearis* but mounted specimens have revealed within them other food bodies such as Euglenoids and diatoms. In its feeding activities, therefore, *A. vorax* appears to be as distinct from the other species as it is in morphological characters.

It may be pointed out that the system of fibrils here described has not been referred to as a neuromotor apparatus. It is doubtful if this term should be used for a set of fibrils that have no obvious connection with the locomotor organs, the cilia. However, there is a certain similarity between the system of fibrils in *Actinobolus vorax* and the system of fibrils described by Rees ('22) for *Paramecium*. One striking point of similarity is the bilaterality of the system, the fibers from each side of the body converging toward different centers. In his rather casual observations on the system of fibrils in *Paramecium*, the writer has come to believe that the distal ends of the fibrils connect with the trichocysts rather than with the cilia, although Rees thought that they connected with both sets of organelles. If their attachment should be found to be limited to the trichocysts, then the similarity between the system of fibrils of *Paramecium* and these inner extensions of the tentacles of *Actinobolus* would be fairly complete.

Summary.

Actinobolus vorax n. sp. is elongate oval to spheroid in contour and usually varies between 100 and 200 microns in length. There are between 30 and 60 longitudinal or slightly spiral rows of long cilia and about 30 tentacles distributed along each row. The cilia are not grouped about the tentacles.

The cytosome at the anterior pole of the body is followed by a pharyngeal apparatus which extends 20 to 25 microns into the body and consists of an inner and outer group of fibrils. These two groups of fibrils converge in the lip where there is a chromophylic circular strand which may be a sphincter or a part of a neuromotor system. There is a large subterminal contractile vacuole at the posterior end and usually a smaller lateral contractile vacuole a little in front of the middle of the body.

The protoplasm is rather uniformly vocuolated, although the central portion is sometimes more uniformly granular. The macronucleus is a long, irregular, rope-like strand, U-shaped, with the free ends in the anterior part of the body. Micronuclei were not definitely identified.

The tentacles are of uniform diameter throughout but contain a toxicyst at the distal end. They may be extended to a length equal to twice the diameter of the body and may be completely withdrawn into the body. They have inner extensions associated with an inner skein of fibrils. The inner extensions of the tentacles of the left side of the body converge to a point at the right side and vice versa. The internal skein is completed by connecting fibrils passing from the right hand center around the body to the left hand center and from the left hand center around to the right-hand center. It is suggested that retraction of the tentacles is accompanied by a winding up process and that extension is accompanied by an unwinding process. It is, on the other hand, possible that the tentacles have extensile and contractile properties within themselves.

In swimming, *Actinobolus vorax* turns on its long axis and describes a spiral path. While swimming the tentacles are withdrawn, but begin to emerge as the animal slows down and become fully extended when the animal comes to rest, as it regularly does with the long axis in a horizontal position. Alternate periods of swimming and quiescence constitute its normal behavior.

Actinobolus vorax feeds primarily on the rotifer, *Anurca cochlearis.* When one of these rotifers swims into the tentacles it stops all movement as if completely paralyzed. It is then drawn near to its captor and passed along to the mouth which opens and engulfs the prey. The same individual may ingest as many as two or three of these large food bodies. In stained specimens of *A. vorax* euglenoids and diatoms have occasionally been seen.

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